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Pesticide Assessment Guidelines, Subdivision O Residue Chemistry. Series 171-4(a)(3) Metabolism (Qualitative Nature of the Residue) Food Animals. Addendum 7 on Data Reporting

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16. Abstract (Limit: 200 words)

The Data Reporting Guideline (DRG) for Metabolism (Qualitative Nature of the Residue): Food Animals, Series 171-4(a)(3), gives guidance to pesticide registrants on the format of their study report so that the Agency can review it efficiently. This guidance clarifies sections in the existing Pesticides Assessment Guidelines, Subdivision O, on data reporting. It provides an outline for the study report and describes the topics which should be addressed such as dosing of radiolabeled materials, housing and health of animals, sample collection, characterization of the radioactivity, etc., and provides guidance on the presentation of the results of the study. Data submitters can use the DRG in preparing their reports for submission to EPA to meet 40 CFR 158 requirements for the registration of pesticides.

The DRG for Series 171-4(a)(3), Metabolism (Qualitative Nature of the Residue): Food Animals is one of a number of DRG's published by the National Technical Information Service as an addendum to Subdivision O of the Pesticide Assessment Guidelines.

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PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION O

RESIDUE CHEMISTRY

Series 171-4(a)(3)

Metabolism (Qualitative Nature of the Residue): Food Animals

ADDENDUM 7 ON DATA REPORTING

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Subdivision O - Metabolism (Qualitative Nature of the Residue): Food Animals

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PESTICIDE ASSESSMENT GUIDELINES

RESIDUE CHEMISTRY

Metabolism (Qualitative Nature of the Residue): Food Animals

Subdivision O, Series 171-4(a)(3)

DATA REPORTING

INTRODUCTION

If a pesticide use could result in residues in edible tissues of livestock, in milk, or in eggs, the registrant is required by 40 CFR 158.125 to submit animal metabolism studies. The purpose of these studies is to determine the qualitative nature of the total terminal residue of a pesticide in the tissues f livestock that have been exposed either by ingestion, dermal use, or premise use. These data are used to determine the "total toxic residue" (the residue considered to be in need of regulation) in tissues, milk, and eggs. Simply put, these studies answer the question, what is the chemical residue?

Animal metabolism studies are discussed in Subdivision O of the Pesticide Assessment Guidelines: Residue Chemistry available from the National Technical Information Service as document PB83-153981. This Data Reporting Guideline (DRG) is supplemental to the Residue Chemistry Guidelines and is designed to aid those who conduct animal metabolism studies intended to support tolerance proposals and pesticide registrations. In particular, this DRG outlines what data are needed to support an animal metabolism study and in what form those data are to be reported. These guidelines will aid the registrant in the collection and organization of data with the goal of developing complete data packages and facilitating the Agency's review of the study report.

When Are Animal Metabolism Studies Needed?

A first step is to determine whether animal metabolism studies are needed. Normally, metabolism studies are required for uses that involve direct treatment of livestock or premise uses. If the use under consideration involves application of a pesticide to a crop, metabolism studies are needed if the raw agricultural commodity (RAC) or a processed byproduct is commonly used as a feed item. The RAC's and their processed byproducts are listed in Table II of the Residue Chemistry Guidelines, cited above. This table also specifies the RAC's considered to be significant feed items, the animals that could ingest them, and the percentage of the total diet that a feed item could comprise.

In certain instances, the petitioner may wish to avoid the question of secondary residues by blocking exposure pathways with label feeding restrictions. These restrictions are appropriate only if they are practical, i.e., the feed item must be in control of the grower (grower control is indicated in Table II of the Residue Chemistry Guidelines), and it must not be of such economic importance that the grower would be likely to feed it even though a label feeding restriction were in place. Corn fodder is an example of a feed item with a high economic importance for which a feeding restriction is not considered practical. Corn

forage and fodder are under the control of the grower, but the high economic value of the feed items and the overwhelming practice of growers to feed these items make the restriction impractical.

This Data Reporting Guideline is designed to aid the petitioner/registrant in generating reports compatible with the Agency's review process. Data submitters are encouraged to submit complete reports for efficient review by the Agency. It pertains to organizing and presenting the substance of the data report. PR Notice 86-5, effective on November 1, 1986 and available from the Office of Pesticide Programs, US EPA, pertains to physical formatting of reports (which are referred to as "studies") and submittal packages. Some of its requirements are mandatory.

RESPONSE TO PUBLIC COMMENTS

The purpose of this section is to acknowledge and address the concerns expressed in the letters of comment received by the Agency in response to the public notice in the FEDERAL REGISTER of March 25, 1988 (52 FR 9536).

This addendum to the Pesticide Assessment Guidelines (Subdivision O) is to be considered an all-encompassing document. The Agency recognizes there are sections in the addendum that do not apply in all cases. Therefore registrants should exercise scientific judgment in deciding which portions are germane to a specific data submission.

This Data Reporting Guideline is not intended to introduce new data requirements or revisions into the Pesticide Assessment Guidelines (Subdivision O) nor is it intended for use by Agency data reviewers as a mere checklist. It is intended to clarify ambiguities in interpretation of existing Guidelines and to organize the submission of data to facilitate the review process.

The Agency has reviewed comments submitted by interested parties. The DRG has been revised in response to several comments. Other comments were considered and in the Agency's opinion, incorporation of these comments would not have improved the efficiency of the review process. Responses to specific comments are as follows:

1. Comment:

   "Table of Contents; The document calls for a specific sequence of report sections (i.e., Introduction and Summary, Materials, Methods, etc.) which differ from those requested in other Data Reporting Guidelines. We suggest the Agency settle on a generic Table of contents for all DRGs."

   Response:

   While several reporting sections are suggested, the given order is not required; the order given follows the usual chronology of a study, but it is not mandatory.

2. Comment:

   "Test facilities: Clarification of the term 'volatile metabolites'

is needed. If it includes $^{14}CO_2$, then the nature of the study changes. This volatile material is a material balance issue which is not critical in this type of study."

Response:

Occasionally it is necessary to show that the label is lost as volatile metabolites, which could include $^{14}CO_2$, because none or very little of the activity is found in tissues or excreta. This would not apply to most metabolism studies.

3. Comment:

The DRG requires listing of sacrifice times to within one hour. "In some cases, the interval from the last dose to sacrifice could differ by more than one hour among those animals in the study."

Response:

This does not usually happen, but if it does, it can easily be resolved by reporting the sacrifice time for each animal. However, for consistency, animals should be sacrificed at approximately the same time after the last dose.

4. Comment:

The proposed tolerance expression is adequately addressed in Sections F/G, and need not be discussed in the report of the metabolism study.

Response:

The discussion of the proposed tolerance expression deals with which residues are in need of regulation, not what the tolerance level should be, which is the subject of section F. The registrant should use the metabolism study to make a suggestion as to which compounds are in need of regulation, i.e., what compounds should be included in the tolerance expression. The DRG has been revised to make this clearer.

5. Comment:

Some commentors took issue with the requirement to provide the "metabolic route for each tissue examined, identity and quantity of significant metabolies, and a proposed tolerance expression." The commentors stated that these studies do not lend themselves to the metabolic route for each tissue, but are limited to describing which metabolites are present. It was suggested that the statement "identity and quantity of significant metabolites identified in each of the major tissues analyzed" be used instead.

Response:

The Agency agrees that requiring the metabolic route is not appropriate. Rather, the procedure used to uncover metabolites should be described

in a schematic diagram, i.e., for each metabolite characterized, the registrant should provide the experimental scheme that led to its characterization. An Agency reviewer is then provided an overview of the experimental details for each metabolite; what it was dissolved in, what chemical treatments were used that may have affected the metabolite, and what physical means were used for its separation. Included in this scheme should be information regarding the relative and absolute amount of each metabolite. The DRG has been appropriately revised.

6. Comment:

"A more detailed explanation is needed from the Agency relative to acceptable criteria for validating storage stability. By definition, classical storage stability can't be done with metabolites that haven't been identified. That's the purpose of the experiment in the first place."

Response:

While classical storage stability studies cannot be conducted for metabolism studies, the Agency requires every precaution be made to ensure that conditions of storage, transit, etc., have not affected the results. These precautions should be documented. In general, frozen storage of less than a month before analysis is acceptable.

7. Comment:

"Listing a series of compounds which were considered as possible metabolites but which were not actually detected, serves no purpose. If an unknown peak is detected and several standard materials are compared chromatographically to it in an unsuccessful attempt at identification, then it would be appropriate to list these standards. However, if the component peak was ultimately identified, this list of what it is not becomes meaningless."

Response:

Such a listing facilitates the review of a metabolism study. It excludes from consideration the listed materials as possible metabolic products which should have been investigated by the registrant.

8. Comment:

"The need for justification and discussion on effects of acid, base, and enzymes on bound/conjugated residues is confusing."

Response:

This is not a requirement, but an opportunity for the conductors of the study to explain procedures that may not be obvious to the Agency. Any unusual techniques or approaches should be explained so that the Agency can provide a fair evaluation of the study. The DRG has been revised to clarify this.

GUIDELINE

The petitioner's report on animal metabolism studies should include discussions of the following topics: the test material, the experimental animals, dosing, sample collection, quantitation of activity, extraction of activity, characterization and identification of activity, conclusions, and raw data.

Often data may be more clearly presented in tables or figures, and included in a separate section. As guidance for when this is appropriate, data requirements that are best submitted as a table or figure are marked with an asterisk (*) in the following guideline.

The following format is suggested for the report.

COVER PAGE

Title page and additional documentation requirements (i.e. requirements for data submission and statement of data confidentiality claims), if relevant to the study report should precede the content of the study formatted below. These current requirements are described in PR Notice 86-5.

TABLE OF CONTENTS

The table of contents should provide page numbers on which are found the essential elements of the study, to include the following: Introduction and Summary, Materials, Methods, Results and Discussion, Conclusions, Tables/Figures, Certification, References, and Appendices.

The requirements of each of these sections are discussed below.

I. INTRODUCTION AND SUMMARY

This section should provide background for the study: It should include the proposed use of the pesticide, the purpose of the study, and a summary of the results.

The summary of the experiment should include a discussion of any unusual problems encountered and how these were resolved, a discussion of any deviation from the experiment's protocol and the effect this may have had on the results, and a brief description of the findings of the study (identity and quantity of significant metabolites in each of the major tissues analyzed, and a proposal as to which metabolites are in need of regulation). A comparison of the results with findings of earlier animal metabolism studies, if any are available, should be included here.

II. MATERIALS

A. Test Substance

1. The test pesticide active ingredient should be identified by chemical name, common name (ANSI, BSI, or ISO), company developmental name or number, and, if available, the Chemical Abstracts Service (CAS) number.

If a metabolite is to be fed, it should be identified by chemical name.

2. If the molecule is labeled in a potentially labile portion or a radioactive atom that is subject to exchange reactions is used, a rationale should be provided. The petitioner should explain their choice for test material, with emphasis on any differences between it and the residue expected in feed items.

3. The impurities in the test material and the potential effect of these on the study should be discussed. The purity of the test material should be reported along with its specific activity in Curies/mole (or mCuries/mmole) and disintegrations per minute per gram (dpm/g).

4. Chemical structures* for parent and metabolites should be provided; each should be accompanied by chemical names and, if available, company developmental name or number.

B. Test Facilities

The animals' housing should be described. For some pesticides for which volatile metabolites are expected to predominate, it will be necessary to establish that volatilization accounts for a significant amount of activity. It will then be necessary to provide a description of the precautions taken to ensure that this activity is detected.

C. Test Animals

A description of the test animals should include their age, weight, health status, and breed. Any health problems or unusual treatment of the animals should be reported; the effect of these on the results of the study should be discussed.

III. METHODS

A. Dosing

1. For oral metabolism studies, the petitioner should describe the preparation of dose (for example, capsule, with feed, bolus, etc.) and the level, timing, and duration of dosing. If the dose is given with feed, the total feed consumed should be reported; the level of pesticide in the feed (determined by counting radioactivity) should also be reported.

2. For a dermal metabolism study, the number, application level, and type of treatment(s) should be described. A comparison of the treatments to those proposed for use on the animals, with particular attention to and explanation of any differences in the formulation, dosing level or other experimental parameter, should be provided.

3. The petitioner should describe the precautions taken to assure that dermally applied pesticide is not orally taken up due to grooming; this is particularly important for ruminants.

B. Sample Collection

1. The petitioner should describe the collection of milk and eggs taken, and provide an explanation if this is different from normal practice.

2. The amount of milk* and number of eggs* should be provided, as well as a comparison of this with normal production.

3. The interval from the last dose to sacrifice should be specified to within one hour. If the animals are sacrificed more than 24 hours after the final dose, an explanation should be provided along with a discussion of the effect of this on the results.

4. A list of the tissues taken and their weights should be provided.* If samples are combined from different animals, this should be stated.

C. Sample Handling and Storage Stability

The storage and handling of samples should be described including the conditions during any shipment and the time in transit. The petitioner should provide evidence that the length or conditions of storage have not significantly affected the results of the study.

D. Analysis of Radioactivity

1. Quantitation and distribution of total recovered radioactivity.

In this section the following should be included:

a. The preparation of the sample prior to counting of activity should be described in detail;

b. The radioactivity recovered in each tissue sampled should be reported as total radioactive counts and in ppm as equivalents of parent compound*; and

c. Counting times*, total counts*, corrected counts*, counting efficiencies*, and other raw data* (sample sizes, sensitivity, limit of detection, etc.) including sample calculations should be reported for representative samples.

2. Extraction and Fractionation of Radioactivity

a. For each tissue the fractionation and extraction strategies should be described by way of a flowsheet*. The solvents used, the order of their use, the extraction procedures (e.g., blending, maceration, soxhlet, etc.) used, and other techniques used to effect extraction should be provided.

b. Any efforts to release bound and conjugated residues (acid, base, or enzyme hydrolysis, exhaustive extraction, etc.) should be

described. The use of severe conditions (e.g., heat plus strong acid) should be justified and the possible effect of these treatments on pesticide residues should be discussed.

c. For each tissue the amount of activity that is water soluble, organosoluble, and unextractable should be reported as a percentage of the total activity in that tissue and in ppm* (parent equivalents).

3. Characterization of Radioactivity

a. A listing* of compounds that were synthesized to serve as standards for known and suspected metabolites should be provided. If TLC, GLC, HPLC or other chromatographic techniques were used to characterize metabolites, appropriate retention times should be provided.

b. Any analytical procedures used to identify metabolites should be described in detail.

c. For each tissue, any losses of activity that occur during the various procedures required for characterization should be explained as fully as possible. This activity should be reported in ppm* (parent equivalents) and as a percentage* of the total terminal activity.

d. For each tissue, any discrete, unidentified activity (e.g., an unidentified spot on a TLC plate) should be reported in ppm (parent equivalents) and as a percentage of the total terminal activity. For each tissue, identified metabolites should be reported in ppm* (parent equivalents) and as a percentage of the total terminal activity. All data supporting the identification (e.g., reproductions of chromatograms and spectra) should be provided. In general, metabolites that comprise greater than 10% of the terminal activity in a tissue should be identified. Failure to identify such a metabolite should be accompanied by an explanation and a description of the attempts that were made to characterize the residue. Any information on the identification and characterization of minor metabolites (less than 10% of terminal activity) should be reported.

All activity should be reported as either:

Free metabolites are normally extractable by organic solvents and do not require chemical treatment to be released.

Conjugate metabolites are those that have been metabolized by the animal to form water-soluble compounds. The conjugate is made up of two parts, that derived from the pesticide, called the exocon, and that from the animal, called the endocon. The endocon is often a sugar, but there are other possibilities (e.g., sulfates, amino acids, glutathione). Identification of the exocon is not normally possible without

cleavage of the conjugate bond. This is normally done by acid, base, or enzymatic hydrolysis. After hydrolysis, the pesticide or pesticide metabolite, free of the conjugating moiety, is usually organosoluble.

Bound metabolites result from pesticide or pesticide metabolites bonding with cellular components to yield products that cannot be removed from the matrix by exhaustive extraction with polar and apolar solvents. If these residues are removed chemically, e.g., by acid, base, or enzymatic hydrolysis, a subclass of bound residues must be established.

Natural constituent is the term applied to a pesticide that has been degraded into small fragments that have been channeled into anabolic cycles and is incorporated into normal cell constituents. If soluble, natural constituents may be difficult to distinguish from conjugates and may be misclassified.

If the natural constituents are unextractable, they are difficult to distinguish from bound metabolites. This may lead to the misclassification of these residues as bound pesticide residues, when they are not pesticide residues at all.

It may be desirable to establish that radioactive residues are natural constituents, particularly if these residues are thought to comprise a large portion of the terminal activity. If needed, there are literature references that will serve as guidelines. They are available in the Standard Evaluation Procedure for Animal Metabolism.[1/]

IV. RESULTS AND DISCUSSION

Residue Characterization: For each tissue of concern (liver, kidney, muscle, fat, milk, and eggs), the petitioner should provide a flowsheet* depicting the significant metabolites and how they were uncovered. The petitioner should also provide discussion of the results including the significance of activity not fully characterized.

V. CONCLUSIONS

The petitioner should reach a tentative conclusion as to the residue in need of regulation including a discussion of whether proposed enforcement methodology will determine these compounds.

VI. TABLES AND FIGURES

NOTE: This section need only include those tables or figures not included in other sections.

1/ To be published by the National Technical Information Service. A Standard Evaluation Procedure describes the manner in which the Agency reviews a study.

A. The following data should be presented in tabular form:

1. Vital statistics of the test animals including, as applicable, weight, milk production, egg production, etc;

2. Level of radioactivity (ppm parent equivalents) in tissues, milk, and eggs;

3. Name, structure, and purity of model compounds used as metabolite standards;

4. Retention times (GC and HPLC) and R_f values for parent and metabolites under the solvent and stationary phase conditions used; and

5. For each tissue of concern (liver, kidney, muscle, and fat) and milk and eggs, the name, structure, and level of all identified metabolites.

B. The following should be presented as figures:

1. Schemes employed for extraction of each tissue;

2. Reproductions of TLC's, GC's, HPLC's, Mass Spectra, autoradiograms, and any other graphic data essential to the conclusions of the study; and

3. Flowsheets describing the significant metabolites in each tissue of concern (liver, kidney, muscle and fat) and how they were uncovered.

VII. CERTIFICATION

Certification of authenticity by the Study Director (including signature, typed name, title, affiliation, address, telephone number and date).

VIII. REFERENCES

Any references cited in the report should be included here.

IX. APPENDICES

Other tables and figures should be included in the appendices, if they are not included elsewhere. Reproductions of published reports or other materials that support the submitted study may also be included in this section, if in the registrants opinion, it will increase the efficiency of the Agency's review of the report.

* Material that would be best presented in a table or as a figure.